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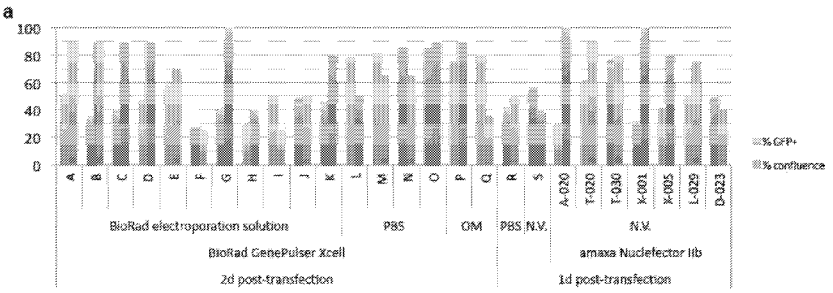
[Continued on next page]

(54) Title: GENETIC CORRECTION OF MUTATED GENES

(57) Abstract: Disclosed herein are transcription activator-like effector nuclease (TALEN)-related compositions and methods of using said TALENs for correcting mutant genes.



b

Program	Conditions
A	1000uF, 120V, 1M cells/200uL, 10ug GFP + 10ug empty vector
B	500uF, 120V, 1M cells/200uL, 10ug GFP + 10ug empty vector
C	300uF, 120V, 1M cells/200uL, 10ug GFP + 10ug empty vector
D	500uF, 100V, 1M cells/200uL, 10ug GFP + 10ug empty vector
E	500uF, 150V, 1M cells/200uL, 10ug GFP + 10ug empty vector
F	500uF, 200V, 1M cells/200uL, 10ug GFP + 10ug empty vector
G	1000uF, 100V, 1M cells/200uL, 10ug GFP + 10ug empty vector
H	1000uF, 150V, 1M cells/200uL, 10ug GFP + 10ug empty vector
I	1000uF, 100V, 0.2M cells/200uL, 10ug GFP + 10ug empty vector
J	1000uF, 100V, 0.4M cells/200uL, 10ug GFP + 10ug empty vector
K	1000uF, 100V, 1M cells/200uL, 10ug GFP + 10ug empty vector
L	960uF, 190V, 0.5M cells/150uL, 5ug GFP
M	950uF, 190V, 1M cells/200uL, 10ug GFP
N	950uF, 160V, 0.5M cells/150uL, 5ug GFP
O	950uF, 160V, 1M cells/200uL, 10ug GFP
P	950uF, 220V, 2M cells/300uL, 10ug GFP
Q	950uF, 100V, 1M cells/200uL, 10ug GFP
R	950uF, 190V, 1M cells/200uL, 2ug pmaxGFP
S	950uF, 190V, 1M cells/100uL, 2ug pmaxGFP

Figure 1

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(88) Date of publication of the international search report:
30 January 2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/38536

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/00, 9/22; A61K 48/00 (2013.01)
USPC - 435/183; 530/300, 350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12N 15/00, 9/00, 9/22, 15/90; A61K 48/00 (2013.01)
USPC: 435/183, 440, 441, 320.1; 530/300, 350; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); ScienceDirect; Google; Google Scholar; IP.com: 'transcription activator,' 'TAL,' 'TAL effector,' 'nuclease,' 'endonuclease,' 'protein,' 'enzyme,' 'dystrophin,' 'mutant gene,' 'stop codon'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	ROUSSEAU, J et al. Endonucleases: Tools To Correct The Dystrophin Gene. The Journal of Gene Medicine. 25 October 2011, Vol. 13, pp 522-537; abstract; page 523, left column, first paragraph; page 536, left column, second paragraph to page 536, right column, first paragraph. DOI: 10.1002/jgm.1611.	1, 2 --- 3/1, 3/2, 4/3/1, 4/3/2, 5/4/3/1, 5/4/3/2, 6/1, 6/2, 8/1, 8/2, 9/8/1, 9/8/2, 10/9/8/1, 10/9/8/2, 11/10/9/8/1, 11/10/9/8/2; 12/8/1, 12/8/2, 16/1, 16/2, 20/16/1, 20/16/2, 36, 37, 38/36, 38/37, 39, 40/36, 40/37, 41/36, 41/37, 43, 44
Y	LI, T et al. Modularly Assembled Designer TAL Effector Nucleases For Targeted Gene Knockout And Gene Replacement In Eukaryotes. Nucleic Acids Research. 31 March 2011, Vol. 39, pp 6315-6325; abstract; page 6315, right column, first paragraph, page 6316, left column, first paragraph; page 6318, figure 1; page 6320, figure 3B. DOI: 10.1093/nar/gkr188.	5/4/3/1, 5/4/3/2, 6/1, 6/2, 11/10/9/8/1, 11/10/9/8/2, 12/8/1, 12/8/2, 20/16/1, 20/16/2, 36, 37, 38/36, 38/37, 39, 40/36, 40/37, 41/36, 41/37, 43, 44
Y	WO 2011/154427 A1 (BOZZONI, I et al.) December 15, 2011; abstract; figure 5a; page 3, lines 7-10; page 6, lines 3-6; page 10, lines 15-20	3/1, 3/2, 4/3/1, 4/3/2, 5/4/3/1, 5/4/3/2, 8/1, 8/2, 9/8/1, 9/8/2, 10/9/8/1, 10/9/8/2, 11/10/9/8/1, 11/10/9/8/2, 12/8/1, 12/8/2

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
15 November 2013 (15.11.2013)

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29 NOV 2013

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/38536

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KUBOKAWA, I et al. Molecular Characterization Of The 5'-UTR Of Retinal Dystrophin Reveals A cryptic Intron That Regulates Translational Activity. Molecular Vision. 07 December 2010, Vol. 16, pp 2590-2597; page 2590, left column, second paragraph, page 2590, right column, second paragraph; page 2591, left column, first paragraph.	16/1, 16/2, 20/16/1, 20/16/2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/38536

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7, 22-35, 42, 45, 46
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Groups I+: Claims 1-6, 8-12, 16, 20, 36-41, 43, 44, SEQ ID NO: 16

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

-***-Continuation of Box No. III - Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: Claims 1-6, 8-12, 16, 20, 36-41, 43, 44, SEQ ID NO: 16 are directed toward a transcription activator-like effector nuclease (TALEN) protein that binds to a dystrophin gene; and a method of correcting a mutant gene in a cell, the method comprising administering to a cell containing a mutant gene a first TALEN and a second TALEN, wherein the first TALEN binds to a first binding region and a second TALEN binds to a second binding region, wherein the first binding region and second binding region are located within a target region and the first binding region and second binding region are not the same, wherein the correction of the mutant gene comprises nuclease mediated non-homologous end joining, and wherein the correction restores the mutant gene.

SEQ ID NO: 16 will be searched without the payment of additional fees. Additional SEQ ID NOs can be searched upon the payment of additional fees. An Exemplary Election would be: SEQ ID NO: 17. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group will result in only the first claimed invention to be searched/examined.

Groups I+ share the technical features including a transcription activator-like effector nuclease (TALEN) protein that binds to a dystrophin gene; and a method of correcting a mutant gene in a cell, the method comprising administering to a cell containing a mutant gene a first TALEN and a second TALEN, wherein the first TALEN binds to a first binding region and a second TALEN binds to a second binding region, wherein the first binding region and second binding region are located within a target region and the first binding region and second binding region are not the same, wherein the correction of the mutant gene comprises nuclease mediated non-homologous end joining, and wherein the correction restores the mutant gene.

However, these shared technical features are previously disclosed by the article entitled 'Endonucleases: Tools To Correct The Dystrophin Gene' by Rousseau, et al. (hereinafter 'Rousseau') in view of the article entitled 'Modularly Assembled Designer TAL Effector Nucleases For Targeted Gene Knockout And Gene Replacement In Eukaryotes' by Li, et al. (hereinafter 'Li'). Rousseau discloses a transcription activator-like effector nuclease (TALEN) protein (page 523, left column, first paragraph; page 536, left column, second paragraph to page 536, right column, first paragraph) that binds to a dystrophin gene (abstract; page 536, left column, second paragraph); and a method of correcting a mutant gene in a cell (abstract; page 536, left column, second paragraph; approximately one-third of DMD patients have a point mutation that results in a nonsense codon and thus in the production of a truncated dystrophin protein, which cannot integrate in the dystrophin complex; specifically engineered endonucleases could be used to treat such patients), the method comprising administering to a cell containing a mutant gene a first TALEN (page 523, left column, first paragraph; page 536, left column, second paragraph to page 536, right column, first paragraph), wherein the correction of the mutant gene (abstract; page 536, left column, second paragraph) comprises nuclease mediated non-homologous end joining (abstract; page 523, left column, first paragraph), and wherein the correction restores the mutant gene (abstract; page 523, left column, first paragraph; page 536, right column, second paragraph; by selecting the right endonuclease targeting the right sequence, it could be possible to induce either micro-deletions including a nonsense codon at the same time as maintaining the normal reading frame or INDELs, which restore the reading frame in a gene with a frame shift mutation). Rousseau does not disclose administering to a cell containing a mutant gene a first TALEN and a second TALEN, wherein the first TALEN binds to a first binding region and a second TALEN binds to a second region, wherein the first binding region and second binding region are located within a target region and the first binding region and second binding region are not the same. Li discloses administering to a cell containing a mutant gene (abstract; page 6315, left column, first paragraph) a first TALEN (abstract; to produce TAL effector nucleases (TALENs) that, in pairs, bind adjacent DNA target sites; we used this approach to engineer 10 dTALENs to target specific loci in native yeast chormosomal genes) and a second TALEN (abstract; to produce TAL effector nucleases (TALENs) that, in pairs, bind adjacent DNA target sites; we used this approach to engineer 10 dTALENs to target specific loci in native yeast chormosomal genes), wherein the first TALEN binds to a first binding region (abstract; to produce TAL effector nucleases (TALENs) that, in pairs, bind adjacent DNA target sites and produce double-strand breaks between the target sequences) and a second TALEN binds to a second region (abstract; to produce TAL effector nucleases (TALENs) that, in pairs, bind adjacent DNA target sites and produce double-strand breaks between the target sequences), wherein the first binding region and second binding region are located within a target region (abstract; to produce TAL effector nucleases (TALENs) that, in pairs, bind adjacent DNA target sites and produce double-strand breaks between the target sequences) and the first binding region and second binding region are not the same (abstract; to produce TAL effector nucleases (TALENs) that, in pairs, bind adjacent DNA target sites and produce double-strand breaks between the target sequences). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have integrated the discovery of utilizing endonucleases, including TALENs, for restoring the function of mutated genes, as previously disclosed by Rousseau, with the use of a pair of TALENs to stimulate non-homologous end-joining, as previously disclosed by Li, since mutant gene restoration is achieved by non-homologous end-joining through the use of TALENs, as previously disclosed by Rousseau, and Li further details the mechanism through which non-homologous end-joining is achieved. Additionally, the use of TALENs to promote the restoration of gene mutations could have benfits in treating diseases involving the dystrophin gene, including muscular dystrophy (Rousseau; page 536, left column, second paragraph; page 536, right column, second paragraph).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the combination of the Rousseau and Li references, unity of invention is lacking.